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First Record of *Cymothoa* sp. Fabricius, 1793 (Isopoda: Cymothoidae) Parasitizing the Four-Eyed Fish, *Anableps anableps* (Cyprinodontiformes: Anablepidae), on the Northern Brazilian coast

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ABSTRACT

This study presents the first record of the occurrence of *Cymothoa* Fabricius, 1793 in northern Brazil, as well as in four-eyed fish *Anableps anableps*. The research was conducted in the Curuçá estuary, in northern Brazil, where 158 specimens of *A. anableps* were collected, with nine *Cymothoa* specimens being found on nine different fish. These isopods were identified by sequencing the mitochondrial Cytochrome Oxidase I gene. The prevalence of the parasite (4.43 %) in *A. anableps* was extremely low and *Cymothoa* sp. specimens were found primarily in the gills of the host fish.

Keywords: Amazon; Isopoda; Crustaceans; Four-Eyed Fish; Anablepidae.

Primeiro registro de *Cymothoa* sp. Fabricius, 1793 (Isopoda: Cymothoidae) Parasitando o peixe de quatro olhos, *Anableps anableps* (Cyprinodontiformes: Anablepidae), na costa norte brasileira

RESUMO

Este estudo apresenta o primeiro registro da ocorrência de *Cymothoa* Fabricius, 1793 no norte do Brasil, bem como no peixe de quatro olhos *Anableps anableps*. A pesquisa foi realizada no estuário de Curuçá, no norte do Brasil, onde foram coletados 158 espécimes de *A. anableps*, com nove espécimes de *Cymothoa,* sendo encontrados em nove peixes diferentes. Esses isópodes foram identificados pelo sequenciamento do gene mitocondrial do citocromo oxidase I. A prevalência do parasita (4.43 %) em *A. anableps* foi extremamente baixa e os espécimes de *Cymothoa* foram encontrados principalmente nas brânquias do peixe hospedeiro.

Palavras-chave: Amazônia, Isopoda, Crustáceos, Peixe com quatro olhos, Anablepidae.

The isopods, the second-largest crustacean order, are one of the dominant ectoparasites of fish (HATA et al., 2017). The genus *Cymothoa* Cymothoa Fabricius, 1793 include 51 species which are all protandric hermaphrodite parasites (JOCA et al., 2015). The species of this genus are widely distributed and may be found in both marine and freshwater environments (AL-ZUBAIDY; MHAISEN, 2013). For South America, 11 *Cymothoa* species have been recorded up to now (LUQUE et al., 2013). In Brazil, the genus is known to occur in the northeast of the country (THATCHER et al., 2003b; THATCHER; FONSECA, 2005; THATCHER et al., 2007; COSTA et al., 2010; CAVALCANTI et al., 2011; ARAÚJO et al., 2012), as well as the southeastern region (SARTOR, 1986; SEVERINO-RODRIGUES et al., 2015). However, there is a lacuna in the data on the occurrence of this genus in northern Brazil (JOCA et al., 2015).

This study provides the first report of ectoparasitism by *Cymothoa* sp. in the four-eyed fish *Anableps anableps* (Linnaeus, 1758) (Anablepidae) and confirms the occurrence of this Cymothoidae in an estuary of northern Brazil. *Anableps anableps* is a viviparous species that inhabits estuarine environments, where it is associated primarily with the intertidal zone of areas dominated by mangroves (OLIVEIRA et al., 2011). This small fish is not commercial interest but plays a vital ecological role in the cycling of the energy produced by mangrove ecosystems (KRUMME et al., 2014).

The present study focused on the estuary of the Curuçá River in northeastern Pará, Brazil (0°37'S, 47°49'W; Figure 1). The Curuçá estuary has a strong marine influence and is dominated by semi-diurnal macrotides that reach 2-4 meters in height (Mácola and El-Robrini 2004). The intertidal zone of this estuary is covered with 116 km^2 of well-developed mangrove forest dominated by *Rhizophora mangle* (L.) and *Avicennia germinans* (L.) Stearn, with some *Laguncularia racemosa* (L.) Gaertn. (Giarrizzo and Saint-Paul 2007; Giarrizzo and Krumme 2008). The local climate is super-humid equatorial, with a mean temperature of 27 °C and mean annual precipitation of 2500 mm, concentrated during the first half of the year (ANA, 2008).

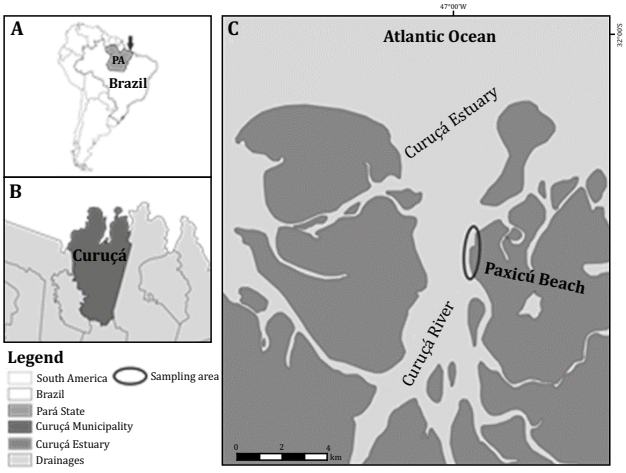

Figure 1. Localization of collection area of *Anableps anableps* in on Paxicú beach in Pará State, Brazil.

The *A. anableps* specimens were collected in the intertidal zone of Paxicú beach, using handheld cast-nets (1.2 cm mesh), in June 2010 (late rainy season), on the quarter moon. Once the fish were captured, their bodies were examined, including the skin, mouth, and gills, to determine the presence of ectoparasites. When encountered, the parasites were removed and conserved in 70 % alcohol. Following the taxonomic identification of the isopods, the position of each parasite on the body of the host was recorded, and its total length (digital calliper, 0.1 mm precision) and total weight

(digital balance, precision of 0.1 g) were determined. The sex of each specimen was also determined based on the presence of an embryo sac on the thorax of the female. The fish specimen was fixed in 4 % formaldehyde for 48 hours, and then rinsed and transferred to 70 % alcohol for storage. The *A. anableps* specimens were then measured (total length, in cm) and weighed (total weight, in g). The the prevalence, mean intensity and abundance were calculated according to Bush et al. (1997).

Samples were taken from one of the legs of each isopod specimen for the extraction of DNA using a Promega extraction kit (SAMBROOK et al., 1989), and a sequence of approximately 600 base pairs (bps) of the mitochondrial cytochrome oxidase subunit 1 (COI) gene was obtained by Polymerase Chain Reaction (PCR), using the universal primers HCO2198 and LCO1490 (FOLMER et al., 1994). The samples were sequenced using the the ABI Big DyeV3.1 protocol (ABI Foster City, USA) in an ABI 3500XL automatic sequencer (Applied Biosystems). The sequences were aligned in BioEdit version 7.1.3 (HALL, 1999) and then compared with isopod sequences obtained from GenBank. The phylogenetic analysis (Neighbor-Joining tree) and p distances were obtained in MEGA 5.0 (TAMURA et al., 2011).

A total of 158 *A. anableps* specimens were collected during the study, with a mean (± standard deviation) total length of 9.65 (± 3.13) cm, and a mean weight of 7.50 g (± 8.24) g. Nine of the specimens (7 females and 2 males) collected were parasitized by isopods.

Nevertheless, the COI sequences identified the isopods as belonging to the genus *Cymothoa*, with a divergence of 2.64 % in comparison with the sequences of *Cymothoa excisa* available in GenBank (Figure 2), further studies are needed to state whether the Isopoda species of the present study is *C. excisa*, due to the few sequences in the GenBank and the use only molecular genetics to the identification of the of this species in the present study. However, this is the first record of this ectoparasite in fishes of the species *A. anableps*. In Brazil, up until now, the only record of *Cymothoa excisa* involved the croaker, *Micropogonias furnieri* (DESMAREST, 1823), a member of the family Sciaenidae, on the coast of the state of Santa Catarina in the southern extreme of the country (THATCHER et al., 2003). Isopods of other *Cymothoa* species have been recorded in Brazil, however, parasitizing fishes of the families Mugilidae, Carangidae, Centropomidae, Gerreidae, Priacanthidae, Sciaenidae, Serranidae, Sparidae, Pleuronectidae, and Ariidae (THATCHER et al., 2003; CAVALCANTI, 2011; ARAÚJO et al., 2012).

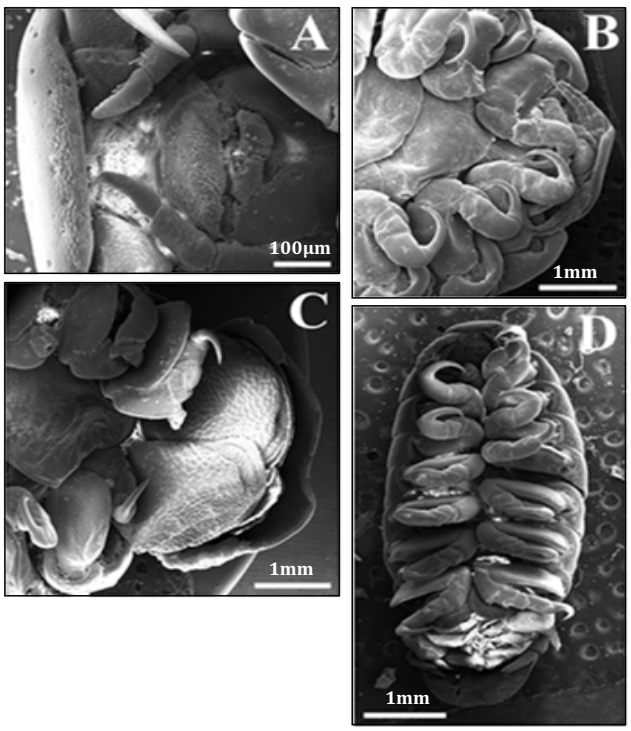

Figure 2. Scanning electron microscope images of ventral views of *Cymothoa* sp. obtained from specimens of *Anableps anableps* collected in the Curuçá estuary in northern Brazil (A = 100 µm; B = 1mm ; C = 1mm and D = 1mm).

The prevalence of *Cymothoa* sp. in *A. anableps* was 4.43 %, while abundance was 0.05, and the mean intensity of parasites per fish was 1.28. A much higher prevalence has been recorded for the genus *Cymothoa*, in the fish *Menticirrhus littoralis* (HOLBROOK, 1847) (20.0 %), for example, and for *C. excisa* (13.0 %) in *M. furnieri* (Thatch#er et al., 2003a; 2003b). The results of the present study thus indicate a low prevalence of *Cymothoa* sp. in *A. anableps*.

One third 33.3 % (n = 3) of the *Cymothoa* sp. specimens were found in the oral cavity of their *A. anableps* hosts, while the other two-thirds (66.7 %; n = 6) were found in the gills of the host. This distribution of isopod parasites is typical of marine fish (COOK, 2012; RAMESHKUMAR; RAMESH, 2016), given that the gills and mouth cavity are well-protected regions with good blood flow and high levels of oxygenation, providing ideal conditions for the survival of ectoparasites (LIMA, 2008).

The *Cymothoa* sp. females were encountered in *A. anableps* with total body lengths of 14 cm (n = 4), 15 cm (n = 2), and 16 cm (n = 1), while the males were found in one individual of 10 cm and a second of 15 cm. The occurrence of the female isopods in larger hosts appears to be related to the capacity for a larger number of ectoparasites (ARAÚJO et al., 2012).

Up until now, little is known of the occurrence of *Cymothoa* sp. in Brazil, in particular in the north of the country, where the genus had not been recorded previously. This is also the first record of *A. anableps* as a host of this isopod. This highlights the need for further research in northern Brazil, in order to amplify the understanding of the diversity of the isopod ectoparasite fauna and its hosts in the Amazon region.

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